

A scientific note on the determination of oxytetracycline residues in honey by high-performance liquid chromatography with UV detection

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To minimize American foulbrood disease (*Paenibacillus larvae larvae*) in honeybees (*Apis mellifera* L.), oxytetracycline (OTC) is used in beekeeping in many countries. OTC can be incorporated into the honey, which therefore becomes contaminated by such residues. This means a potential risk of toxic or allergic reactions for hypersensitive people (World Health Organisation, 1969). For this reason, a simple method for the detection of OTC residues in honey by high performance liquid chromatography (HPLC) coupled with ultraviolet-visible spectrophotometry using a photodiode array detector (HPLC-DAD) has been developed according to the Council Directive 96/23/EC. In the European community, the use of antibiotics is not allowed in apicultural practice. Due to this reason, no limit has been fixed for maximum residue as in other food products like milk and muscle, where the limit is 100 µg/kg.

A sample of honey (5 g) was dissolved in 20 mL of 0.1M Na₂EDTA-McIlvaine buffer at pH 4 (Oka et al., 1987; Mascher et al., 1996). The sample solution was stirred for 5 minutes, filtered and loaded on a Waters OasisTM HLB 3cc (60 mg) cartridge previously conditioned with 1 mL of methanol and 1 mL

of water. The SPE cartridge was then washed with 10 mL of water and the sample was eluted with 1 mL of ethyl acetate directly in 1 mL volumetric flask. The SPE cartridge chosen for the extraction and clean-up operation showed good performance in terms of time, recovery and solvent consumption. After evaporating the solvent at 40 °C under nitrogen stream, the volume of 1 mL was reconstituted with the HPLC mobile phase.

The HPLC analysis was carried out using a Luna 5 µm phenyl-hexyl column (150 × 4.6 mm I.D.) (Phenomenex, Torrance, CA, USA) in isocratic conditions with methanol-acetonitrile-0.01M aqueous oxalic acid (2:3:16) mobile phase (Oka et al., 1987; Diaz et al., 1990). The flow rate was 1.0 mL/min. The detector monitored the eluent at 360 nm and measured spectra from 200 to 400. The sample injection volume was 10 µL. In the HPLC chromatogram, OTC resulted as a peak at *t*_R 6.6 minutes. Figure 1 shows a representative chromatogram of a blank honey sample and of honey spiked with 1 µg/g of OTC.

The linearity of the HPLC assay was checked by analysing a series of honey samples fortified with oxytetracycline standard solutions in the

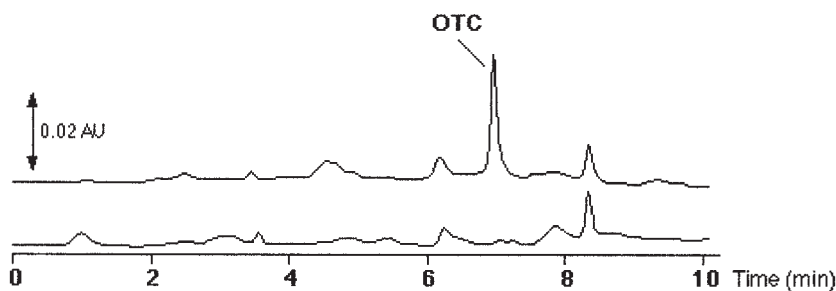


Figure 1. Comparison of HPLC chromatograms: 1 µg/g OTC spiked honey (upper profile); unspiked sample (lower profile).

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concentration range 0.25 to 13 µg/mL. The calibration curves obtained using the linear least squares regression procedure of the peak area versus concentration exhibited good linearity ($r = 0.998$). The precision of the assay, calculated on 20 samples fortified, expressed as the relative standard deviation was 1.7%. The recovery of three concentration's levels of 50 µg/kg, 100 µg/kg and 500 µg/kg and the relative standard deviation (s_r), obtained with 6 assays each, was 82% (3%), 87% (5%) and 85% (3%), respectively. The limit of detection (LOD) and quantification (LOQ), estimated as those concentrations of analyte which yield a signal-to-noise (S/N) ratio respectively of at least 3/1 and 5/1 (De Ruyck et al., 1999), were 25 µg/kg and 50 µg/kg.

The method tested on commercial samples of honey of different botanical origin showed good versatility and rapidity. Currently it is routinely used in the laboratory of National Institute of Apiculture (accredited according to EN 45001) to evaluate the risk of OTC contamination.

Note scientifique sur la détermination des résidus d'oxytétracycline dans le miel par chromatographie liquide haute performance avec détection UV.

Eine wissenschaftliche Notiz zur Rückstandsbestimmung von Oxytetracyclin im Honig durch Hochdruckflüssigchromatographie mit UV Detektor.

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